Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY Inventors: Alexander Gaiger et al. Serial No. Not yet assigned Docket No.: 210121.465C5

HU: MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPPGASAYGSL MO: MGSDVRDLNALLPAVSSLGGGGGGGCGLPVSGAAQWAPVLDFAPPGASAYGSL

HU: GGPAPPPAPPPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTVHFSGQFTGTAG MO:GGPAPPPAPPPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTLHFSGQFTGTAG

HU: ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYSTVTFDGTPS MO: ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYSTVTFDGAPS

HU: YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG MO: YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG

HU:SQALLLRTPYSSDNLYCMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTE MO:SQALLLRTPYSSDNLYCMTSQLECMTWNQMNLGATLKGMAAGSSSSVKWTE

HU: GQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDVRRVPGVAPTLVRSAS MO: GQSNHGIGYESDNHTAPILCGAQYRIHTHGVFRGIQDVRRVSGVAPTLVRSAS

HU: ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR MO: ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR

HU:SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR MO:SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR

HU: WPSCQKKFARSDELVRHENMHQRNMTKLQLAL MO:WHSCQKKFARSDELVRHENMHQRNMTKLHVAL



FIG. 2

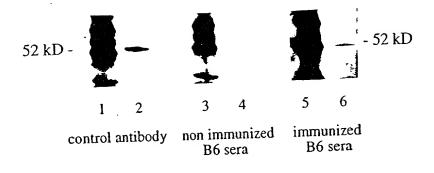


FIG. 3

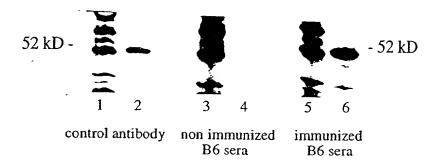
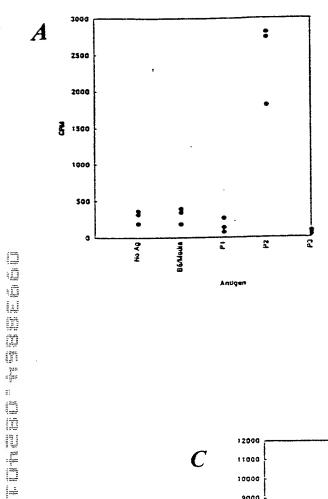
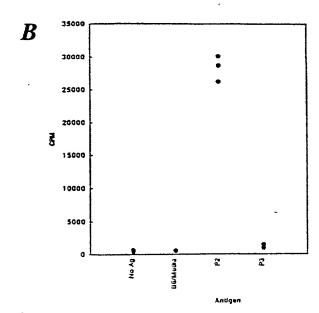


FIG. 4

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

Docket No.: 210121.465C5 Inventors: Alexander Gaiger et al. Serial No. Not yet assigned





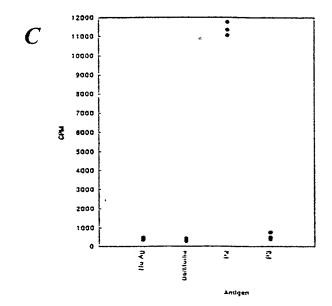


FIG. 5A-5C

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY
Inventors: Alexander Gaiger et al. Serial No. Not yet assigned Docket No.: 210121.465C5

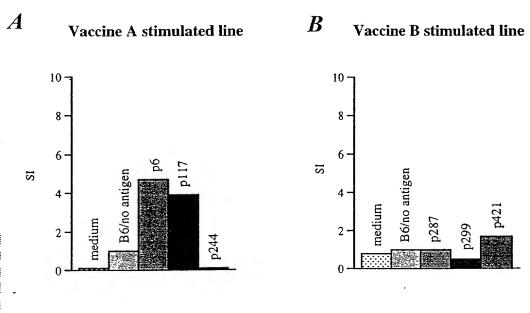


FIG. 6A and 6B

A STATE OF THE PERSON NAMED IN COLUMN NAMED IN

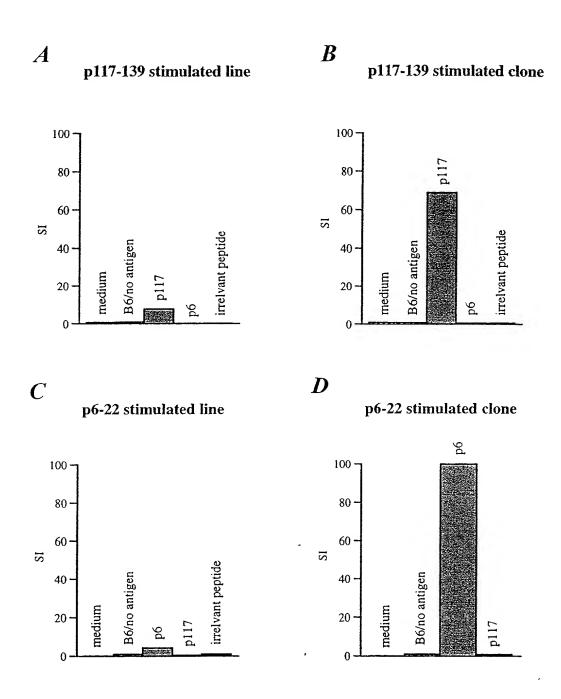
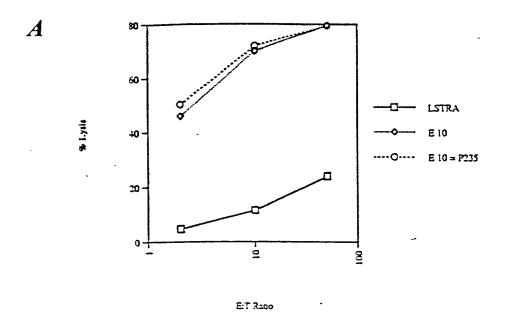


FIG. 7A-7D

5		15		25									70	15
MGSDVRD	LNALI	PAVPS	LGGG	GGCAL	PVSGA <i>I</i>	QWAP'	VLDFA	PPGAS	AYGSL	GGPAP	PPAPP	PPPPP	PPHSF:	IKQE
AA	AAAAA	AAAAA	AAAA	A	AA	AAA.		AA	AAAAA	AAAAA				
• • • • • • •														
				• • • • • ·		• • • •	• • • • •	• • • • •	• • • • •	• • • • •	• • • • •	• • • • •	• • • • •	
80	85	90	95	100	105	110	115	120	125	130	135	140	145	150
PSWGGAE	PHEEC	CLSA	TVHE	SGOFT	STAGAC	RYGP	FGPPP	PSOAS	SGOAR	MFPNA	PYLPS	CLESQ	PAIRN(	QGYS
• • • • • • • •														
												• • • • •	• • • • •	
155	160	165	170	175	180	185	190	195	200	205	210	215	220	225
TVTFDGT	PSYGE	TPSHE	IAAOF	PNHSFE	KHEDPN	IGOOG:	SLGEO	OYSVP	PPVYG	CHTPT	DSCTG	SOALL	LRTPYS	SSDN
			· • • •	• • • • •		· · · ·		• • • • •		· · · · ·	• • • • •		• • • • •	• • •
													295	
LYCMTSC	LECMI	MNQMN	ILGAT:	LKGVAZ	AGSSSS	VKWT:	EGQSN	HSTGY	ESDNH'	TTPIL	CGAQY	RIHTH	GV FRG	QDV
AAAAAAA	.A		A.	AA.AA	١							AA	<b>LAAAA</b>	<i>KAAJ</i>
			RRR	RRRRRR	RR	.333	яя						RRRR.	
DDDDDDD.														
						• • • •			<i>.</i>	• • • • •		aaaaa		
				205	220	225	2.0	246	250	25-	7.00	265	270	275
													370	
RRVPGVA														
AAAAA														
RRR	RR					RRRR			<i>.</i>					
	DDDDD	D												
300	205	300	305	400	405	410	4.5	420	125	430	435	440	445	450
RHTGVKE														
	. <b></b>	. <b></b> .	.ddd	dddddd	ddd									,

	5	10	15	20		30						60		70	
MGSD	VRD:	LNALL	PAVSS	LGGG	GCGL	PVSGA	ACMAP!	VLDEA.	PPGAS.	AYGSL	GGPAP	PPAPP!	PPPPP	PPHSFI	KQE
	.AA	AAAAA	AAAAA	LAAAA	A	AA/	AAAA.	• • • • •	AA	AAAAA	AAAA	• • • • •			• • •
• • • •	• • •			• • • •	· · · · ·	RRI	RR	• • • • •		• • • • •			• • • •	 	• • •
• • • •	• • •	• • • • •	• • • • •	• • • •	• • • • •		• • • • •	• • • • •	• • • • •	• • • • •	• • • • •				
• • • •	• • •	• • • • •		• • • •			• • • • •								
8	0	85	90	95	100	105	110	115	120	125	130	135	140	145	150
PSWG	GAE	PHEEQ	CLSAF	TLHES	SGQFT	STAGA	CRYGP:	EG222	PSQAS	SGQARI	MEPNA	PYLPS	CLESQ	PTIRNÇ	GYS
					AA	<b>λ</b> Α	· • • •	• • • • •		AA	A	AAA	AAA		• • •
					I	RRRR.	• • • • •	• • • • •		RI	RRRR.	• • • • •			• • • • •
• • • •	• • •	• • • • •		• • • •				• • • • •	DDDDD	. פטטס	· • • • •	• • • • •	• • • • •		• • •
• • • •	• • •	• • • • •	• • • • •	• • • •		• • • • •	• • • • •	· · · · ·	• • • • •	• • • • •			• • • • •	· · · · · ·	• • •
15	. 5	160	165	170	175	180	185	190	195	200	205	210	215	220	225
TVTE	DGA	PSYGH'	TPSHH	AAQF!	PNHSE	KHEDP!	MGQQG	SIGEQ	QYSVP	PPVYG	CHTPT	DSCTG	SQALL	LRTPYS	SDN
			A	AAAA							AAAA	Α		<i>.</i> .	. AA
			R	RRR.							· · · · ·				
										• • • • •		DDD	טטטטט	DDDDDD	
	• • •	• • • • •		• • • •			• • •			• • • • •	• • • •				• • •
27	ın	235	240	245	250	255	250	255	270	275	280	285	290	295	300
T.YOM	T SO	LECMT	WNCMN	LGAT	LKGMA	AGSSS:	SVKWI	EGQSN	HGIGY	ESDNH	TAPIL	CGAQY:	HTHIR	GVFRGI	QDV
EZZA	בבב	Δ .		A	AA.AA	A							AA	<u> </u> ፍ <u>ጸ</u> ፈጸፈል	AAA
				.RRR!	RRRRR	33	333 -	2		• • • • •		• • • • •		RRRR	• • •
DDDD	. מס	• • • • •	• • • • •	!	ופססססו	יכפספספ	0	• • • • •		• • • • •			 ddddd		
• • • •			•							• • • • •			aacaa		
3.0	۱۲	310	315	320	325	330	335	340	345	350	355	360	365	370	375
RRVS	GVA	PTLVR	SASET	SEKR	PEMCA	YPGCN:	KRYFK	LSHLQ	MHSRK	HTGEK.	SĀĞCD	EKDCE	RRFSR	SDQLKF	HQR
AAAA	ιA	AAAAA	AAAAA	ιΑ				:				A	AAA.A	AAAAAA	AA.
	RRR	RR					.RRRR	· · · · ·		• • • • •			• • • • •		• • •
DE	DDD	ססססס	D							• • • • •					
• • • •	· • •	• • • • •			<del>-</del>			• • • • •							
3.5	10	385	390	395	400	405	410	415	420	425	430	435	440	445	450
внто	:VX5	FOCKT	CORKE	SRSD	HLKTH	TRIHT	GKTSE	KPFSC	RWHSC	QKKFA	RSDEL	VRHHN	MHQRN	MTKLHV	AL
			AAAA	AAA.	AAA		AAAA.			AA	. AAAA	AAAA.	AAA	A	
									· · · · ·	RRR	RRR	RR	• • • • •		• •
								• • • • •	• • • • •	• • • • •		• • • • •			••.
				ddd	adddd	adad.		• • • • •	• • • • •	• • • • •		• • • • •			• •





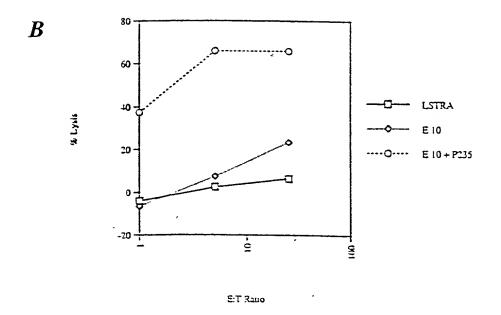


FIG. 9A and 9B

80

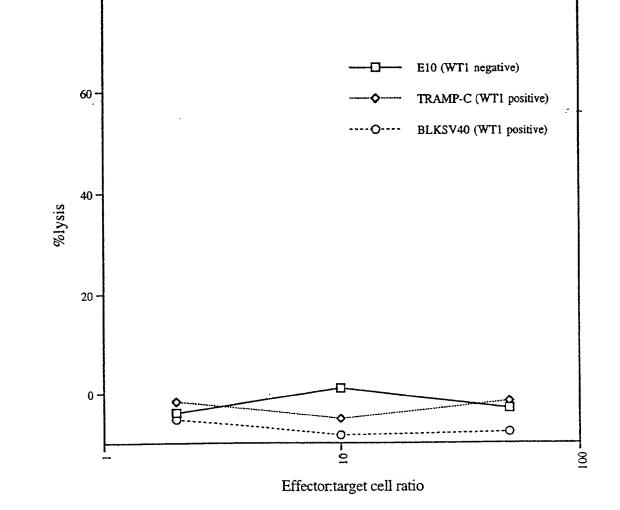
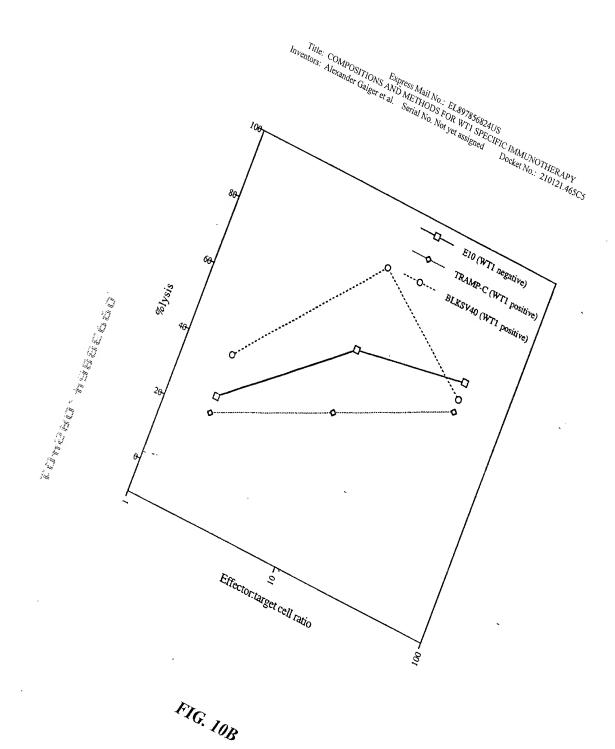


FIG. 10A



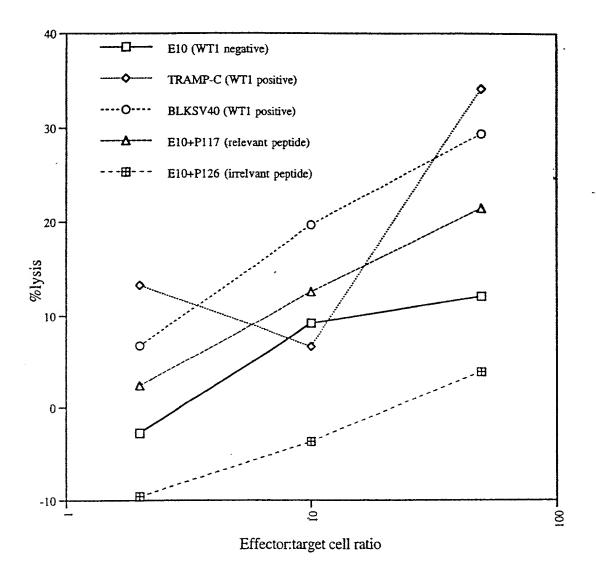
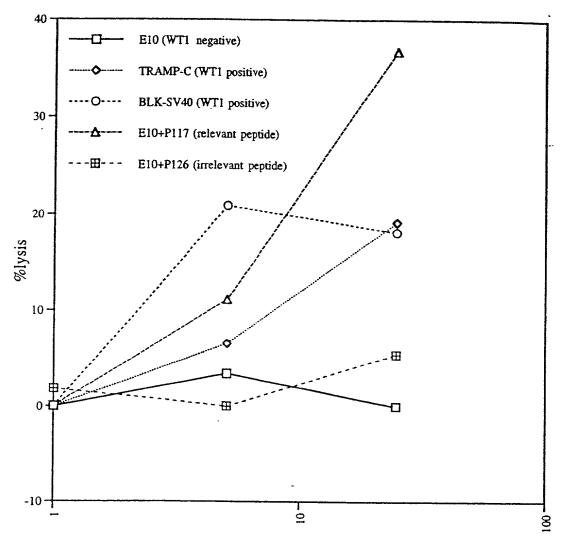


FIG. 10C

 $k_{j}$ 

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY
Inventors: Alexander Gaiger et al. Serial No. Not yet assigned Docket No.: 210121.465C5



Effector:target cell ratio

FIG. 10D

1/

Company to the District Company of the State of the State

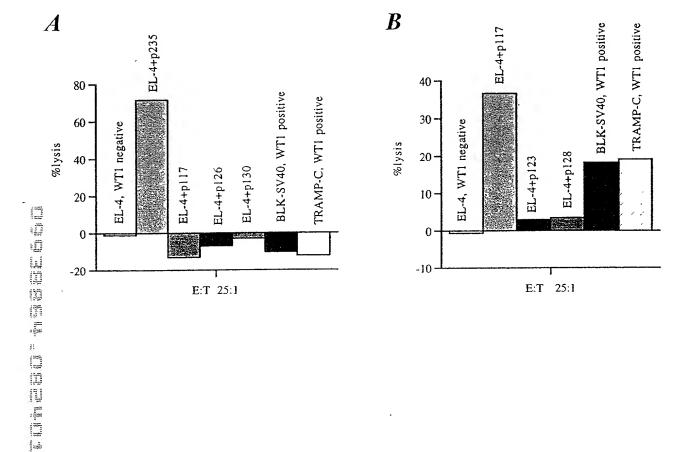


FIG. 11A and 11B

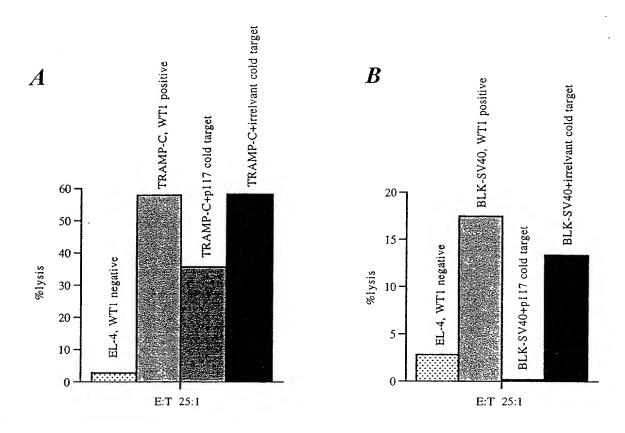
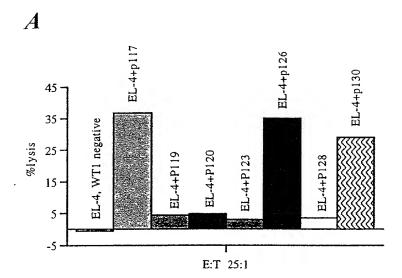


FIG. 12A and 12B



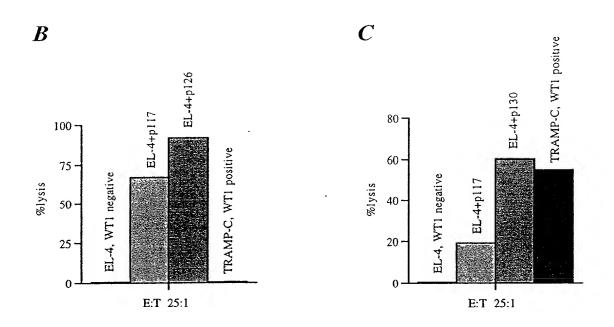
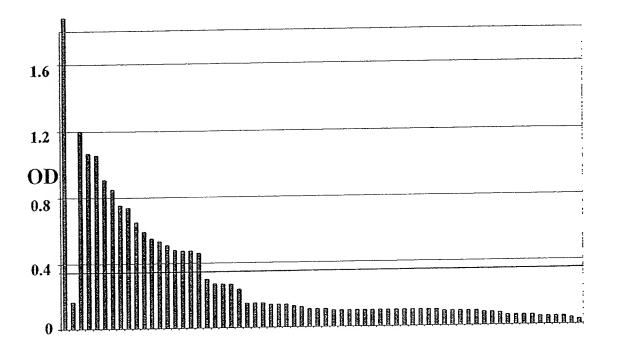
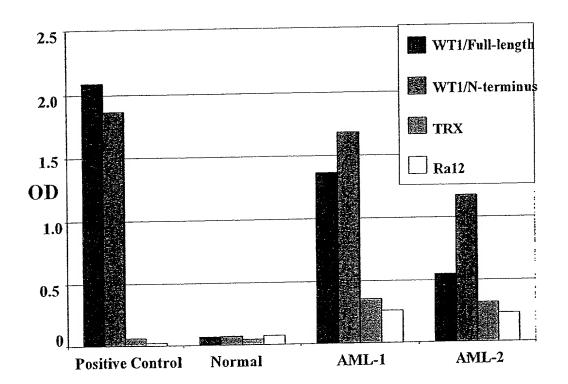


FIG. 13A-13C



F19. 14



F19.15

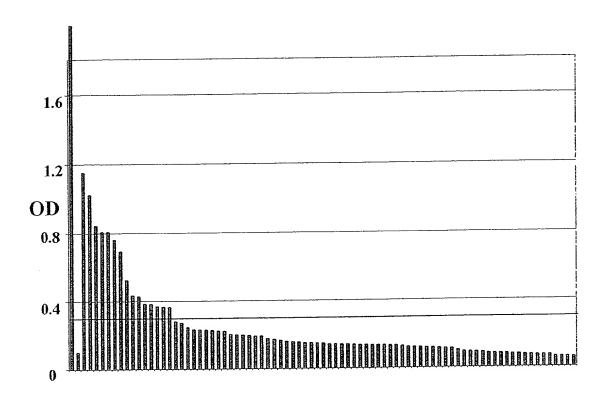
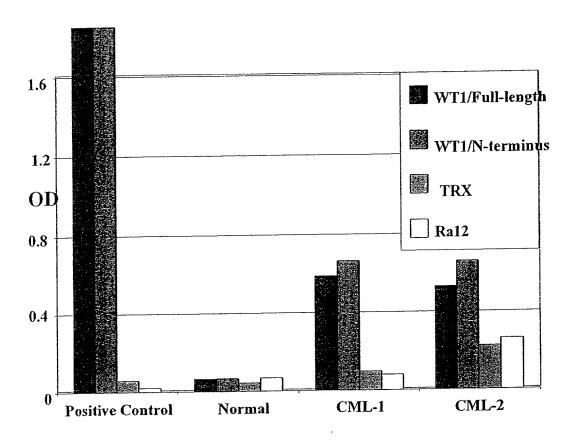


Fig. 16





F19.17

TABLE 1: Characteristics of Recombinant WT1 Proteins Used for Serological Analysis

Molecular Weight	85kDa	60kDa	50kDa
WT1 Aming Agid Bosition Molegular Weight	aa 1-449	aa 1-249	aa 267-449
Decombinent Drotein	Ral2-WT1 fill length fission protein	TRX-WT1 N-terminus fusion protein	WT1 C-terminus protein
	WT1/fill-lenoth	WT1/N-terminus	WT1/C-terminus

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18D 18C 18B 18A 17D 1.7C 1.7B 1.7A 1.6D ■ WT1A/TRX ■ RA12/WT1 16C 1.6B **DRA12** o TR3 TRX 16A 1.5D 15C 15A 13D 13C **Mouse Titration** 13A **9** 1.2D **8** 12C **8** 12B 1.2A 1.1D 11B 11A .4D .4C 4B .4A .3C .3B .3A .tD .tC .iB ŧΑ WT180 0.0 0.8 Optical Density o ni density 0.2 0.7 0.3

CID000622 Figure 1a Ab responses in group 0 and 1 (controls)

The first first first far for first first first first

I CH THE THE THE AND THE

Control groups. A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

FIG. 19 A

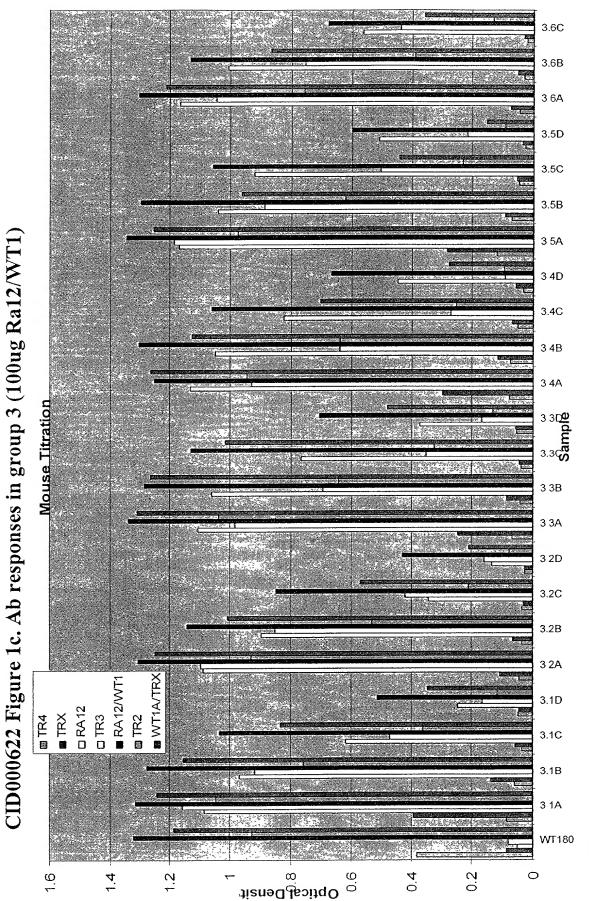
2 8C 2.8B 28A 2.7D 2.7C 2.7B 2.7A 2.6D CID000622 Figure 1b. Ab responses in group 2 (25ug Ra12/WT1) 2.6C 2 6B 2 6A 2.5D 2 5C 2 5B Mouse Titration 2.5A 2 4Ddws 2 4Ddws 2.4C**S** 2 4B 2.4A 2 3D 2 3C 2 3B 2.3A 2 2D 2.2C 2.2B ■ RA12/WT1 **■** WT1A/TRX 22A 0 RA12 2.1D a TR3 TR4 21C 2.1B 2 1A WT180 6  $\sup_{\dot{\omega}} \mathbf{Densit}_{\dot{\omega}}$ 0.2 4.0 0

25ug Ra12/WT1+MPL-SE, A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

28D

FIG. 1973

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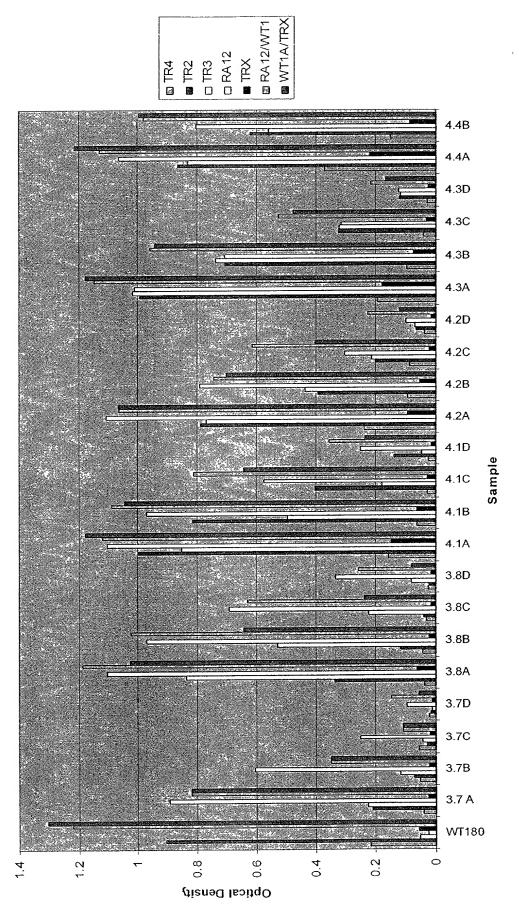
ngagaga ngbuni

WT1. Dose Titration. Ab responses to WT1. 100ug Ra12-WT1+MPL-SE. A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

TIG. 190

Express Mail No.: EL897856824US
Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY
Inventors: Alexander Gaiger et al. Serial No. Not yet assigned Docket No.: 210121.465C5

CID000622 Figure 1d. Ab responses in groups 3 and 4 (1000ug Ra12/WT1) **Mouse Titration** 



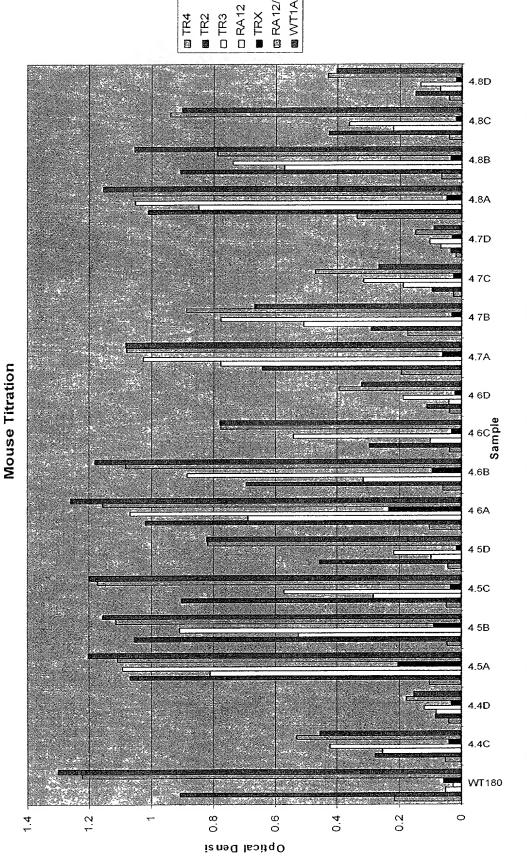
WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE. A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

TIG. 19D

Express Mail No.: EL897856824US
Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY Docket No.: 210121.465C5

Inventors: Alexander Gaiger et al. Serial No. Not yet assigned ■ WT1A/TRX ☑ RA12/WT1

Figure 1e. Ab responses in group 4 (1000ug Ra12/WT1)



WT1. Dose Titration. Ab responses to WT1. 1000ug Ra12-WT1+MPL-SE. A: 1;500 Dilution, B: 1:2000, C: 1: 8000, D: 1:16000

TIG. 19 E

Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY Inventors: Alexander Gaiger et al. Serial No. Not yet assigned Docket No.: 210121.465C5

Figure 2a. Proliferative T-cell responses in WT1 protein immunized mice. (Ra12WT1 dose titration, 3x in vivo, after 2IVS)

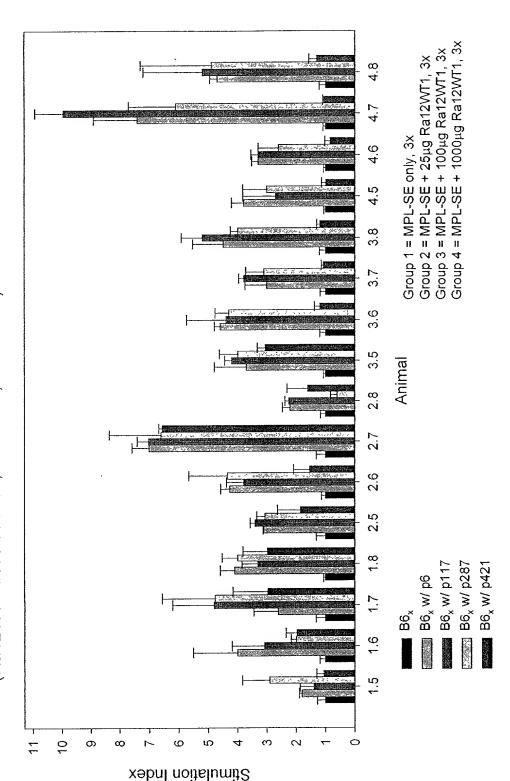


FIG. 20 A

Figure 2b. Proliferative T-cell responses in WT1 protein immunized mice (Ra12WT1 dose titration, 6x in vivo, after 2IVS)

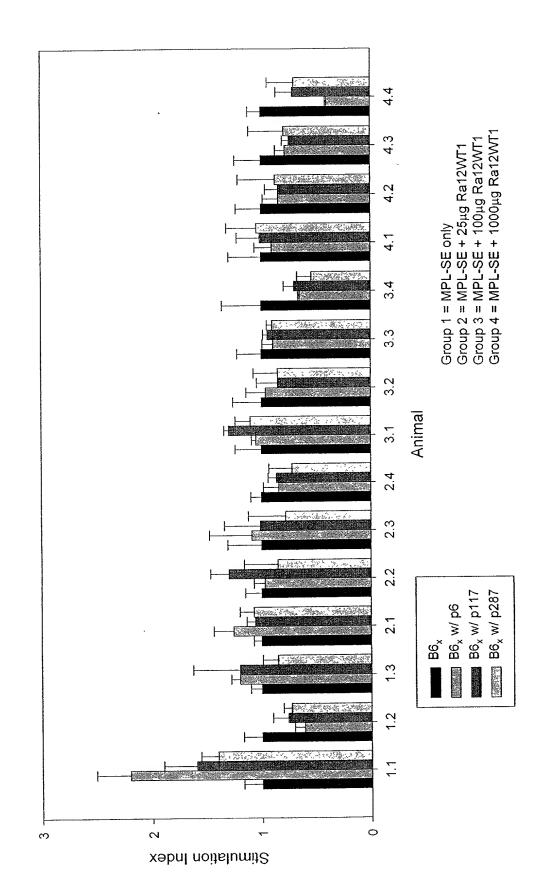


FIG. 20E

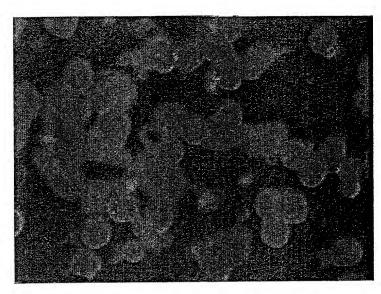
Express Mail No.: EL897856824US
Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY

Inventors: Alexander Gaiger et al. Serial No. Not yet assigned

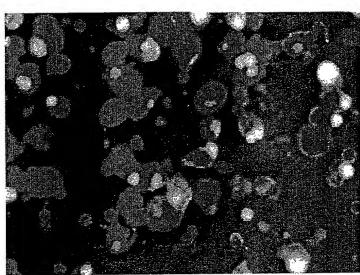
Docket No.: 210121.465C5

## FIG. 21 Figure 1. WT1 expression in human DC following adeno WT1 and Vaccinia WT1 infection

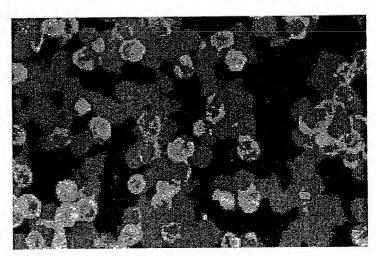
**Control** (uninfected human DC



Adeno WT1 infected human DC



Vaccinia WT1 infected human DC

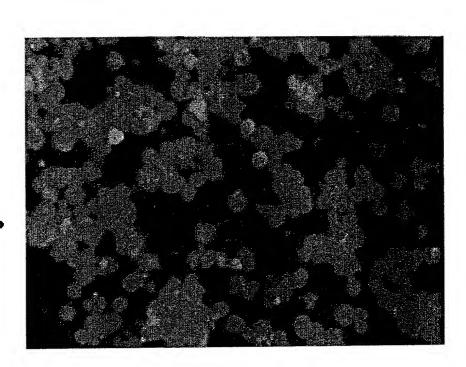


Title: COMPOSITIONS AND METHODS FOR WT1 SPECIFIC IMMUNOTHERAPY Inventors: Alexander Gaiger et al. Serial No. Not yet assigned Docket No.: 210121.465C5

FIG. 22

Figure 2. WT1 can be expressed reproducible in human DC following adeno WT1 infection and is not induced by a control Adeno infection

**Control** (Adeno EGFP infected human DC)



Adeno WT1 infected human DC

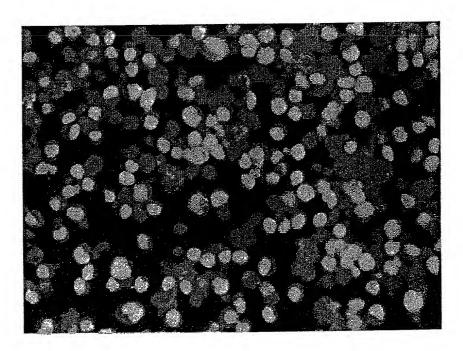


FIG. 23

